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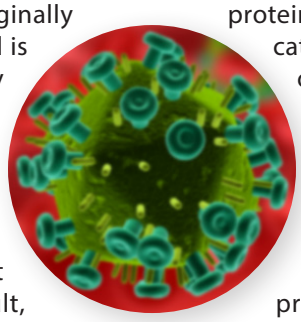
Trends & Developments in BioProcess Technology

A Production of BioProcess Technology Network

From The Editor-In-Chief:

I've had some very interesting technical issues cross my desk in the last month, and one involves the "state-of-the-art" production technology for lentiviral vectors. And while you'd think this process should be quite advanced since the industry has been manufacturing viral gene vectors for over 20 years, it appears that we've fallen back on old technology and we're dealing with processing issues we would typically avoid.

I've been told that the vast majority of the lentivirus vector applications involve the use of multi-plate, plastic devices such as Cell Factories™ (Nunc/Thermo Fisher) which must be kept at culture temperature in a warm room, or via other measures. Then the media is recirculated through the various plates as it is automatically adjusted for pH and dissolved oxygen. The 293T (originally 293tsA1609neo) is the cell line of choice, and is one derived from a human embryonic kidney (HEK 293) cell line that can stably express the SV40 large T antigen. Then the 293FT cell line, which is yet a further derivative, is said to produce lentiviral vectors at even higher levels. But for now, the 293T cells are anchorage-dependent in a process that requires fetal bovine serum (FBS). As a result, this very valuable viral vector, a key ingredient in the CAR T cell process, is being produced with 30 to 40 year-old viral vaccine technology.



My first question: If these cells must be grown attached to a surface, why aren't roller bottles being used, or certainly packed bed bioreactors, or even microcarriers in a stirred tank system? And since single-use systems are so popular, why not grow the 293T cells on microcarriers in bags on a rocker platform? Then I remembered that lentivirus is being produced in an iCellis® packed bed bioreactor, which is now distributed by Pall Life Sciences. However, I haven't found any data on lentivirus production with the Fibra-Cel® disks typically used in the packed bed systems from Eppendorf, which I thought would be a more likely technology—especially in academia.

I can't help but think back to when adenovirus was a popular gene vector originally produced with anchorage-dependent HEK 293 cells using FBS. But as quickly as they could, process developers adapted the cells to suspension and eliminated the serum. Then lower and lower protein media formulations were developed which still resulted in high virus yields. So why wouldn't we be looking at a lentivirus process with 293T cells that are grown in suspension without animal-derived serum? And what does this say about a lentivirus reference material that would need to be meaningful for a considerable period of time? If we're going to end up with lentivirus produced without animal serum proteins, how representative would a lentivirus preparation be in which serum proteins could remain after clarification and purification, and how would such a material affect the outcome of very sensitive assays?

Then via an incredible review article written by Otto Merten (Généthon) *et al.*, I discovered that considerable progress has been made in adapting stable cell lines to suspension while producing attractive lentivirus yields without FBS, and with increasingly lower amounts of protein. And while production in hollow fiber bioreactors has its limitations, this and other technologies have shown potential.

Now I'm not claiming to have done extensive literature and interview research, but my assertions can't be too far off. My primary purpose is to stimulate thinking about what the lentivirus process will be in the near future, and what the resulting virus micro-environment will be like to work with. And how will we effectively compare virus produced with the current technology with material produced with technology that I believe will soon become dominant? Maybe it doesn't matter. However, this is a biologic for which the "product is the process." I can't help but think that we should be making comparability decisions with viruses that are produced with a technology we know the industry will be adopting.

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